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THE CHROMOSOME VIEW OF HEREDITY AND ITS MEANING TO PLANT BREEDERS[1]

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DEFINITE advice as to practical procedure must be based on a firm foundation of fact if the leaders in the applied science are to retain any confidence in those who lay the first stones in the pure science. At the same time, if it is clearly understood that science only approximates truth, that so-called "established laws" are only highly probable and never absolute, it can hardly be said to be unwise if an inventory of fact is taken at any time. The handwriting on the wall is never finished; some words are dim and the erasures and omissions are many, but that is no reason why one should not try to read it and to see what it directs if he has translated aright.

This preliminary justification of the title of this article is made because our present stock of facts regarding heredity points clearly to the chromosomes as vital parts of the mechanism, and I wish to emphasize some important practical deductions in case this position continues to become more firmly established.

A just and complete dissertation upon the rôle of the

[1] This paper is based upon two lectures delivered at Harvard University in 1914. I hope that any cytologists who may have their attention called to it will overlook the repetition of some well-known facts in the first few pages, as it is intended to be merely a general statement of a particular point of view with certain deductions that follow if it be accepted. I wish to thank Doctors O. E. White, T. H. Morgan and R. Goldschmidt for their kindness in giving me many suggestions, but in justice to them I should state that they are not responsible for the conclusions drawn.

chromosomes in heredity not only would fill many pages, but would expose numerous gaps in our present knowledge, gaps that leave several important questions in the balance. We shall assume frankly therefore that the chromosomes *are* the bearers of the determiners of practically all of the hereditary characters that have been investigated by pedigree culture methods, acknowledging freely our ignorance on many points, but maintaining that while no facts have been discovered which offer insurmountable arguments against the viewpoint taken, the following logical sequence of truths discovered at various times and by different methods of research make a pretty sound case upon which to base our practical conclusions.

RELATIVE IMPORTANCE OF NUCLEUS AND CYTOPLASM

There are several reasons for believing that of the two parts of the cell, the nucleus and the cytoplasm, the former plays the greater rôle in heredity.

In general it is believed that the two parents contribute equally in the production of offspring—that the male and female contribution of potential characters is practically the same. If there were a difference it would be shown by divergent results in reciprocal crosses, but the investigations following Mendel's method make it probable that with the exception of sex and sex-linked characters, the results of reciprocal crosses are generally alike. This being true, it would appear that the principal basis of inheritance must be sought elsewhere than in the cytoplasm, for in most observed cases the sperm is very much smaller than the egg, and this difference is largely a difference in the amount of cytoplasm each carries. Is one not to look for some significance in this disparity in size? Strasburger, as well as other botanists, has even gone so far as to declare the male generative cell in certain angiosperms to be simply a naked nucleus that slips out of its cytoplasmic coat into the embryo sac, leaving the discarded coat behind, and that stimuli proceeding from the nucleus control the assimilation of food in the cell and determine even the character of the cytoplasm itself.

This belief may be too radical. The machine must have all of its parts to do proper work; and it may be, as Conklin suggests, that such characters as polarity, symmetry and localization of organ bases in the egg have their chief seat in the cytoplasm. This is only a possibility and not a fact, however, for one must admit that cytological investigation has not disclosed the presence of a material basis of heredity in the cytoplasm, though he may not be convinced that it is unimportant. Does the same statement hold for the nucleus?

The nuclear cavity contains four substances as they are ordinarily described in connection with morphological investigations. These are nuclear sap, linin, nucleolar material and chromatin.

Nuclear sap probably belongs as much to the cytoplasm as to the nucleus, and we know nothing as to its possible significance and importance within the nucleus.

Linin by some investigators is regarded as very similar to chromatin. Others (Strasburger) consider it to be the framework of the chromosomes, and the only real substance within the nuclear cavity that is continuous from generation to generation. It is a thread-like material staining lighter than chromatin upon which the chromosomes appear to be strung in the early prophases of nuclear division.

Nucleolar substance, though it stains in a different manner from chromatin, is considered by many to be chromatin-like in its nature. It is the substance of which the nucleoli are composed; but as these bodies become vacuolated and finally disappear during nuclear division, one is led to believe with Strasburger that they are temporary storehouses of some necessary food material.

Chromatin, however, as the material of which the chromosomes are composed, plays such a peculiar part in the activities of the cell, that hypotheses as to the meaning of its behavior are certainly more than shrewd guesses, as will be seen.

The chromosomes may be described as morphological

elements, of various shapes and sizes that are found within the nucleus; they are especially demonstrable as deeply staining bodies, definite in number for each cell at the period of division. In many cases in both plants and animals they have been found to be made up of small particles, the chromomeres, and various investigators have expressed the belief that these, too, are definite in number and play an important part in the larger collective entity, the chromosome.

Almost from their discovery, the chromosomes have had an especially important part assigned to them in the drama of heredity because of the previous philosophical deductions of Weismann. Weismann reasoned that if there were no reduction of heritable substance in the life cycle of an organism, it would pile up indefinitely because of the nuclear fusion at fertilization. He, therefore, predicted the discovery of some mechanism by which the character conserving substance would be divided. A few years later his prediction was verified in its important details by actual observation of the chromosome reduction in the formation of germ cells in *Ascaris*. From this discovery and from the facts that a specific number was found for the cells of each species, that all the cells of an individual appeared to possess the same number (except when they were halved at gametogenesis), that they were apparently permanent organs, that they were longitudinally halved in division so as to give each daughter cell the same number as well as an exact half of each chromosome possessed by the mother cell, investigators were early tempted to place upon chromosomes the whole burden of inheritance.

Our observations regarding chromosomes and the reduction divisions in plants now rest on a basis of cytological investigation of over 250 species, representing over 150 genera and divided among the four great groups of this kingdom. Montgomery's 1906 list of chromosome numbers in animals represents investigations on 185 species, comprised in about 170 genera, distributed among

nearly all the phyla of the animal kingdom. Sex chromosome studies have undoubtedly increased these figures for the animal kingdom to date, by hundreds of species.

Variation in chromosome number among the cells of an individual plant or animal is a recognized fact among cytologists, but this variation is not regarded as of particular significance, as commonly it is held to exist only among old cells, cells highly specialized, or, at any rate, cells which will never have anything in common with reproduction. To quote from Strasburger,

> the number of chromosomes in the nuclei of the somatic cells of both the sexual and the asexual generations have been found to vary. But so far as my experience goes, these observations are always to be observed in the nuclei of cells which are no longer embryonic, like those in an embryo or growing point, but which, on the contrary, are to some extent histologically specialized and are not destined eventually to give rise to reproductive cells. The determinate number is still more frequently departed from in nuclei which are definitely excluded from the sphere of reproduction.

In the reproductive cells, chromosome division is, on the other hand, very exact, and the numbers found, almost invariable, with one exception. This exception is the so-called accessory chromosome or chromosomes, that appear to be coupled with sex differentiation. And the very fact that such accessory chromosomes do exist and by their presence or absence parallel sex distribution, forms one of the most unanswerable arguments in favor of the chromosomes being the chief bearers of character determinants.

Morphological Individuality of the Chromosomes

The next topic to consider is whether there is sufficient evidence to support the idea that these bodies—the chromosomes—are morphological entities persisting from one cell generation to another.

Prochromosomes are deeply staining bodies found in the resting cell nuclei of plants, which probably correspond in number, but not in size, to the chromosomes which are found in the dividing nuclei. These bodies are

thought to represent the resting nuclear condition of the chromosomes. Prochromosomes have been found in at least sixty species of plants, and various structures comparable to them in many others. These investigations favor the thought that the chromosomes are persistent morphological entities; nevertheless they are not sufficient to establish the matter if there were no other data at hand.

There is a series of facts, however, which is more convincing. We are told that in addition to each species of animal or plant having in the larger part of its cells a specific number of chromosomes, there is a constant reappearance of the different shapes and sizes of these chromosomes in the same positions relative to one another during cell division after cell division.

Strasburger says: "The observation of such a series of stages of nuclear division as can be obtained by the laying open of embryo sacs in which development of endosperm tissue is commencing, makes it difficult to resist the impression that it is always the same chromosomes which make their appearance over and over again in the repeated divisions. In the prophase, the chromosomes are seen to appear in precisely the same position that they occupied in the preceding anaphase, and if the picture of the anaphase were proportionally enlarged, it would exactly correspond to that of the succeeding prophase."

The facts from which these general conclusions have been drawn can not be denied. Baltzer found odd-shaped chromosomes of similar shape in many maturing eggs of sea urchins. Boveri, Montgomery and later Schaffner pointed out a constant difference in the form and the size relations of the two chromosomes of *Ascaris megalocephala univalens*. Sutton thought he could recognize each individual chromosome in eleven consecutive cell generations of the maturing germ cells of the lubber grasshopper *Brachystola magna*. The so-called sex chromosome which has been found in so many insects and

other animals, is a clear case of constancy in appearance. In plants the same phenomenon has been observed. Rosenberg investigated the pollen mother cells of *Crepis virens* and in certain stages in division invariably found two long, two intermediate and two very short chromosomes. Division figures in the somatic cells showed the same differentiation, and in an examination of the nuclei of the pollen grain he found only one chromosome of each kind present. Such other species of this genus as have been investigated also show some variation in chromosome form, although it is not so striking as in *C. virens*. *Hieracium venosum*, exceptionally good material also investigated by Rosenberg, has shown the same thing. Edith Hyde remarks on the fact of the constant reappearance of certain chromosome forms among hundreds of division figures which she observed in *Hyacinthus orientalis*. Sauer mentions a very long chromosome constantly present in pollen mother cell preparations of the lily-of-the-valley, and Strasburger and Lutz found a large chromosome among many small ones in *Lychnis dioica*. In certain species of *Yucca* this chromosome differentiation takes on a dimorphic aspect, ten of the chromosomes being very large and about forty-five very small.

Taking into consideration all of these facts, of which hardly more than a random sample has been given, one is clearly justified in concluding that these cell characters are reproduced generation after generation. Why this constancy if they are not important?

Physiological Individuality of the Chromosomes

There is also considerable reason for believing that the various chromosomes of a cell may have different functions.

Boveri was the first to endeavor to test this hypothesis by allowing sea-urchin's eggs to be fertilized by two spermatozoa. Three nuclei, each with eighteen chromosomes, were thus present in the same egg, two male and one female. Although cytoplasmic division seemed to pro-

ceed normally, the chromosomes were usually distributed irregularly by a three-poled or a four-poled spindle. As a result three or four cells were produced at the first division of the doubly fertilized egg, instead of the two cells that arise after normal fertilization. Various abnormal larvæ were produced later. In such embryos, Boveri found the organism to be divided into definite regions, thirds or fourths, each part traceable to one of the three or four original cells, and the cells of each part differing from the cells of the other parts in their combination of chromosomes and usually in their chromosome number. In rare cases normal embryos were produced, but these were more commonly developed from a doubly fertilized egg which in its first division was three-celled, than from one in which it was four-celled. The thought occurs at once that three cells have a better chance than four cells in securing a full set of chromosomes, both as to number and kind. If the division were normal, each nucleus would receive a full set in the case of the chromosome distribution to three cells, but the division is usually irregular, and because of this irregularity each cell does not usually secure its normal set of chromosomes. Nevertheless it is clear that the embryo parts developed from the three-celled cleavage stand a much greater chance of being normal than those from the four-celled type, although through irregularities in division an eighteen-chromosome-celled region might be formed even where the first division was four-celled.

In some cases, the embryo was completely normal as regards skeleton and pigmentation in one or even two of its thirds, while the remainder was entirely lacking in these characters. Nearly normal embryos occurred which were perfect as to parts and specific characters, but individual variations which normally should have appeared in separate larvæ were present among the thirds. Asymmetrical larvæ also were formed.

More important still are the results Boveri obtained by isolating the three cells of the three-fold type and the

four cells of the four-fold type and allowing them to develop into larvæ. When the four cells of a four-celled stage of a normal embryo are separated, each cell produces a normal dwarf embryo alike in every respect, but the three- or four-celled embryos from double fertilized eggs, when treated in the same manner, never produce normal dwarfs even when the chromosome distribution has been numerically equal. Large numbers of larvæ brought into existence through this experiment showed all possible combinations of characters, just as all possible chromosome combinations were found in their nuclei, and from these and other data the conclusion is drawn that "not a certain number, but a certain combination of chromosomes is necessary to normal development, and this clearly points out that chromosomes have different qualities." In other words, the sea urchin has a set of eighteen chromosomes, each chromosome performing at least some different functions from its neighbors, making it necessary for the whole set to be present in order to insure normal development.

In further investigations, Boveri placed sea-urchin eggs which had been normally fertilized and were about to divide under pressure. As a result, division of the nucleus took place, but often no division of the cytoplasm. Such eggs on again dividing often formed more than two poles, resulting in inequalities in chromosome distribution and abnormal larval development. Boveri puts upon these cases an interpretation similar to that of the preceding experiments, as the irregular chromosome distribution seems to be all they have in common.

Morgan comments on Boveri's experiments as follows:

> The evidence makes probable the view that the different chromosomes may have somewhat different functions and that normal development depends on the normal interactions of the materials produced by the entire constellation of chromosomes.

Artificial parthenogenesis and experiments with enucleated eggs have proved that only one set of chromosomes is necessary to normal development of embryos, but it is

important, in considering these experiments, to note that two sets of similar chromosomes are present in a normal sexually produced organism.

Pairs of chromosomes of each shape and size (if they differ in shape and size) are *nearly* always found in the somatic cells—the exception being when the so-called accessory chromosomes are present. And since but one of each kind is found in the two gametes that fuse to form the new organism, it is only natural to suppose that one set was contributed by the maternal parent and the other by the paternal parent.

The numerous cases in which this phenomenon has been demonstrated are to many the most convincing evidence of some sort of a morphological individuality of the chromosomes. To them the fact implies pairs of freight boats loaded with the essential materials of life, to others—the minority—it is no more wonderful than the constant recurrence of other plant organs. At any rate, it has been shown that these sets of chromosomes continue an apparently independent existence for some time. Moenkhaus produced hybrids between the two species of fish, *Fundulus heteroclitus* with long straight chromosomes and *Menidia notata* with short curved chromosomes, and the early divisions of the fertilized egg showed clearly complete sets of chromosomes from each parent. Rosenberg obtained similar results in crosses between the two sundews, *Drosera longifolia,* which has forty small chromosomes, and *Drosera rotundifolia,* which has twenty large chromosomes. In some cases similar to the latter, where one parent contributes a greater number of chromosomes, it should be noted that the organism seems to have regulatory powers. The chromosomes unnecessary for a double set are either thrown out or take no part in the activities of cell division. For example, in the supposedly hybrid sundew, *Drosera obovata,* Rosenberg found that its thirty chromosomes behaved in the following peculiar manner. Ten of them paired with another ten, but the other ten remained unpaired and acted in a very abnormal fashion

in the reduction divisions. The ten pairs separated normally, one of each pair going to each pole; but the ten unpaired were irregularly distributed, sometimes nearly all of them going to one pole, sometimes most of them becoming lost in the cytoplasm and forming small nuclei. Embryos were produced in a very few cases and these only through back-crossing with pollen of *D. longifolia*. Unfortunately these embryos only developed through a few cell divisions.

These chromosome pairs have been distinguished by the name homologous chromosomes. For a long time it was thought that the paternal and the maternal set of chromosomes separated from each other bodily at the reduction division. Now it is believed to be only a matter of chance which chromosome of a pair passes to a particular daughter cell. There is some cytological evidence for this view, but the main argument in its favor is that this behavior is all that is necessary to fit nearly all the known facts of heredity, with the chromosomes playing the part of the active heredity machinery as will be seen shortly. This statement is true in a broad sense, but the word nearly is used because there is an exception to it. Chance apportionment of either member of a homologous pair of chromosomes to a daughter cell accounts for all facts of alternative (Mendelian) inheritance except where there are breaks in the correlation between characters usually inherited together. Since such breaks in correlation are common, it is clear that there must be a period when chromosome pairs have such an intimate relation that material can be exchanged. Many biologists believe that such a period is found during the maturation of the sex cells. The particular point at which such a conjugation or approximation of chromosome pairs takes place is called synapsis; it occurs as a part of the prophase or first stage of the reduction division. Some investigators have been unable to demonstrate any real chromosome fusion at this time, but all agree that there is an approximation between the two sets, and a chance for some kind of an exchange or interaction to take place.

Evidence of the physiological individuality of the chromosomes may be concluded by referring briefly to the so-called accessory chromosome. This fraction of a chromosome, whole chromosome, or in some cases, group of chromosomes, possesses no true synaptic mate, and therefore at reduction division two types of daughter cells are found. The presence or absence of the "accessory" is so closely associated with sex determination that most biologists now regard it as the morphological expression of a germinal sex determinant. The essential result of researches on this body may be summed up in the following words of Wilson.

> They have established the existence of a visible difference between the sexes in respect to these chromosomes, and have shown that it is traceable to a corresponding difference in the nuclei of the gametes of one sex or the other.

The simplest type of accessory chromosome, where the male possesses an unpaired chromosome which passes to one pole undivided in one of the spermatocyte divisions and hence enters but half the spermatozoa, was discovered by Henking (1891) in *Pyrrhocoris*. This work was confirmed in certain species of Orthoptera in 1902 by McClung, who advanced the hypothesis that the odd chromosome was a sex-determiner. Shortly afterward this was made more probable by Wilson and by Stevens who proved for several species of Hemiptera that the body cells of the males contain one less chromosome than the females. Two accessory or X chromosomes are present in the female, while but one is present in the male.

About the same time, both Wilson and Stevens independently discovered another kind of dimorphism in male germ cells of certain Hemiptera. Here the X chromosome of the male has a smaller synaptic mate Y. The body cells of the female, however, show two of the large X chromosomes. The sexes, therefore, both contain the same number of chromosomes, but have the same type of chromatin difference as was first discovered. The female is XX and the male XY.

Baltzer claimed in 1909 that in the sea urchins *Sphærechinus* and *Echinus* the sex with the dimorphic germ cells is the female instead of the male, but the work of Tennent has shown him to be in error and he has retracted the statement. There is, therefore, no undisputed cytological evidence demonstrating this type of dimorphic eggs; but since breeding results on certain species of birds and of lepidopters can be interpreted only on such an assumption, it is safe to assume that sooner or later they will be found.[2] Whether or not there are animals of this type, however, is of no particular importance in the present discussion. What we desire to emphasize is that a large number of animals, including man, have been shown to have a chromatic difference between the sexes, and that this difference is readily explained by the fact that the eggs are of a single type and the spermatozoa of two types.

In diœcious plants no such morphological differentiation has been found. But this fact does not negate the idea that the visible differences found in animals are really sex-determining differences. We have only to suppose that the dimorphism is primarily qualitative and secondarily quantitative. Indeed Wilson has found that the Y chromosome—the synaptic mate of the X—may vary in different species from a size equal to that of X until it disappears entirely, leaving X without a mate.

There is only one criticism in this whole matter. One may admit these cytological differences between the sexes, but hold that they are early appearances of secondary sexual characters. Morgan, von Baehr and Stevens have answered this impeachment. In the phylloxerans and aphids all the fertilized eggs produce females; males arise only by parthenogenesis, though females may arise in this manner. The cytological facts are as follows: Under favorable external conditions eggs develop without reduction and females are formed. Under unfavorable conditions one or two chromosomes (the sex determiners) are thrown out. If these eggs develop without fertilization

[2] Dimorphic eggs in Lepidoptera have recently been demonstrated by both Doncaster and Seiler.

males arise. The somatic condition of the females may therefore be termed XX and that of the males XY. If both reduced normally at any time, ordinary fertilization might be expected to give both males and females. But the spermatocytes without X degenerate, leaving only one type of functional spermatozoa, which produces females. Thus actual causal connection between the X chromosome and sex determination appears to have been demonstrated.

These are the main cytological arguments in favor of the chromosome view of heredity that seem to me to be insuperable. There are minor arguments both pro and con, which, as I said in the beginning, we have not space to consider. Instead it seems more profitable to show how Mendelian results interlock with those from cytology like the parts of a jig-saw puzzle.

CHROMOSOMES AND MENDELIAN INHERITANCE

The principal phenomena of Mendelian inheritance are: (1) characters that breed true; (2) uniformity of the population of the first hybrid generation in particular traits in which homozygous parents differed; (3) independent segregation of certain character determiners; (4) recombination of certain characters; (5) perfect coupling between certain characters; and (6) partial coupling between certain characters. Let us see how plausibly one can picture the mechanism through which such phenomena may result without imputing to the chromosomes any behavior that is not known to occur. To do this simply let the imagination portray a plant species having four chromosomes, each chromosome having three character determinants that can be followed through the breeding results that are obtained.

Our figures represent the immature germ cells of the plant just previous to the reduction division. Fig. 1 shows the germ mother cell with a duplicate set of hereditary determinants. The mature germ cells are exactly alike, therefore the plant breeds true to the characters concerned.

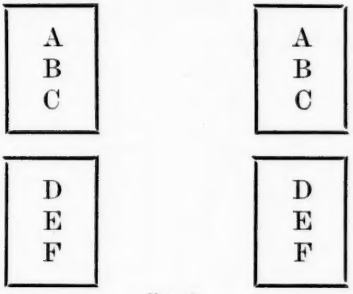

FIG. 1

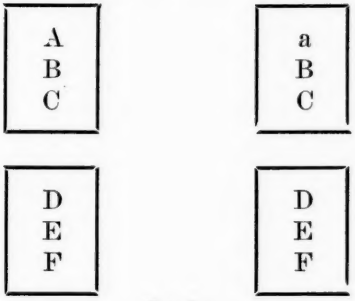

FIG. 2

Suppose, however, that a change in the germ plasm has occurred (Fig. 2) at some time or other. In one member of the first pair of chromosomes, determinant "A" has become "a." The mature germ cells differ from each other by one factor. For this reason the plant does not breed true, but gives a mono-hybrid Mendelian result.

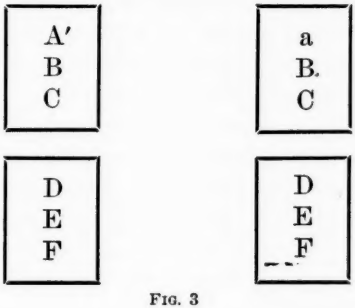

FIG. 3

Again, if such a change occurs that A becomes A′ (Fig. 3), a series of triple allelomorphs giving monohybrid results with each other, is formed. "A" is allelomorphic to "A′" or "a."

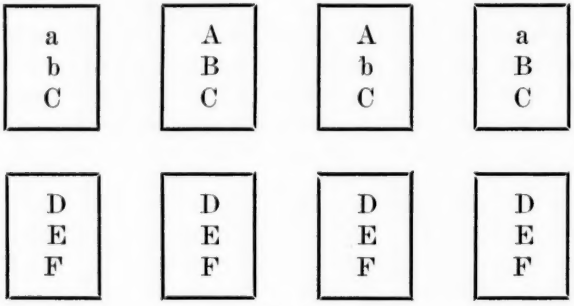

FIG. 4

But there are other character determinants in the first pair of chromosomes. What happens if both "A" and "B" become changed? There are two possibilities, as shown in the two parts of Fig. 4. If one of the members of the pair of homologous chromosomes becomes abC while the other remains ABC, there is a positive correlation between the inheritance of "A" and "B." On the other hand, if the change is such that the two chromosomes are aBC and AbC, there is a negative correlation between A and B. In other words, the determinants remain correlated in the same way they entered the combination. There may be breaks in these correlations, however, as Morgan has shown in *Drosophila;* and these breaks in correlation occur in a constant ratio. Diagrammatically, it may be said that A and B are always the same distance apart in the chromosome structure and that the determinants "cross over" from one member of a pair to the other every so often. All of the gametes in the first case are not ABC and abC, for example. Some of them will be AbC and aBC. And the same percentages of these cross overs are found in the second case where "A" and "B" are correlated negatively. Furthermore,

if C should become c, and the chromosome pair take the form ABC and abc, there are definite relations between the three determinants. Breaks in correlation occur, and this ratio is constant, so that if given the percentage of breaks of correlation between "A" and "C" and "B" and "C," the percentage of breaks between "A" and "B" can be predicted. If there is a break in the correlation between "A" and "C" 30 times in 100, and a break between "B" and "C" 10 times in 100, then there will be breaks in the correlation between "A" and "B" 20 times in 100.

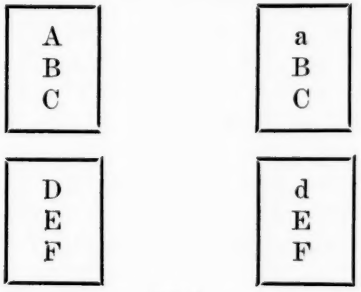

FIG. 5

Likewise, the determinants in the second pair of chromosomes are coupled together in their inheritance. D, E and F have each their peculiar linkage to the other, a linkage that remains comparatively constant. Yet the determinants in the second pair of chromosomes are entirely independent from those in the first pair in their inheritance. For example, if, as shown in Fig. 5, "A" should become "a" in either member of pair number one, and "D" should become "d" in either member of pair number two, Mendelian dihybridism would result. Furthermore, if "A" and "D" should each have the function of affecting the same general character complex in somewhat the same manner, there would be an apparent 15:1 ratio if dominance were complete or a series of types ranging from the type of one grandparent to that of the other, if dominance is lacking.

These are the main features that have been established

by recent work on hybrids. We have *pictured* them as actual chromosome functions, because every part of the description has been actual fact as far as the breeding experiments go. Our picture, it is true, is fictitious, for we do not know absolutely that the heredity mechanism is of this nature. But the facts do fit perfectly all that is known of chromosome behavior. It seems impossible, therefore, that there should be so many coincidences.

There are also two other pieces of evidence that fit in and round out the case. Bridges has shown that females occasionally occur in *Drosophila* bearing the sex-linked characters borne by the mother but showing no influence of those borne in the father. Such exceptional females were found to inherit directly from their mother the power of producing like exceptions, and it was proven cytologically *after the prediction had been made from the breeding facts* that these females resulted from the non-disjunction of the X chromosomes at the maturation of the eggs from which they came, and that one half of their daughters did in fact contain a Y chromosome in addition to two X chromosomes. This appears to be definite proof that sex-linked genes are borne by the X chromosomes.

The other important basis for regarding the chromosomes as the material basis for heredity also comes from Morgan's work on *Drosophila ampelophila,* this being the only species upon which sufficient work has been done to give a reasonable basis for the conclusion. *All of the hundred and thirty or so mutations in this species upon which Morgan and his students have worked are so linked together in heredity that they form four groups corresponding to the four pairs of chromosomes found in the species. If one single character should be found that did not fit into one of these four groups, the whole theory would break down. But no such character has appeared.*

This completes the case for the chromosomes as regards the main facts, and it seems only proper that a fair-minded jury of scientists should render verdict for the plaintiff. No case is so bad, however, that a lawyer can

find nothing to say for the defense and scientists in this respect resemble the men of the bar. Certainly there are some outlying facts, but they are comparatively unimportant. If a series of important facts should at any time be found which do not fit, the chromosome mechanism should be looked into. It is likely that the explanation will be found in an abnormal chromosome behavior as was the case in the aphis.

Practical Conclusions and Discussions

If now it be accepted as a reasonable premise that the chromosomes are the chief if not the sole bearers of hereditary determinants of body characters, and that their behavior is a rough indication of the mechanism of heredity; what cytological facts, if any, can be made useful at present or in the future to plant and animal breeders? If such data exist, they should be put to service; if it is likely that such facts can be found, investigations should be undertaken. The broad question may be divided into three parts which will be discussed in regular sequence:

1. What are the relations of chromosomes to somatic characters?
2. What are the relations of normal chromosome behavior to the transmission of characters?
3. What are the relations of peculiar or unusual chromosome behavior to the transmission of characters?

Relations of Chromosomes to Internal Characters

Some very interesting observations have been made on the relations of internal and external characters to chromosome number.

Farmer and Digby in a comparative study of the cells of a fern of the genus *Athyrium* with similar cells of three of its varieties, found that the measurements were successively larger in the three varieties than in the species, and that there was a corresponding increase in the number of chromosomes, the gametic numbers for the species and its varieties being estimated at 76–80, 84, 90 and 100,

respectively. Investigations on another fern, *Lastrea,* did not corroborate these results, however, in one variety the chromosomes being more numerous and the cells smaller than in the parent type.

Gates by comparing nuclei and cells of different tissues of *Œnothera Lamarckiana* and similar structures in its "mutant" *O. gigas* with double the number of chromosomes, found that the *O. gigas* cells and nuclei were always larger, varying from a comparative ratio of 1:1.5 to 1:3. At the same time, it would hardly be wise to maintain that this is always the case, for only a few individuals were investigated.

Primula sinensis has two forms in cultivation, similar except as to size. The giant form has flowers about one and one half times the size of those produced by the ordinary form. Gregory investigated these two forms cytologically to determine the cause of this increase. The nuclei and the chromosomes of the giant form were a little larger, though the difference was hardly a measurable one. The chromosome number was the same in both the forms. In a later investigation he has found that some exceedingly large plants with nuclei distinctly larger than those of the normal form had double the number of chromosomes normal to the species.

Boveri investigated this same relation of cells and nuclei to chromosome number in N, 2N and 4N larvæ of the sea urchin. From these studies, he concludes that chromatin is non-regulatory, and in the case of decrease, unregenerable, the cytoplasm in contrast showing the fullest regulatory activity. Further, the size of the larval cells is governed by the chromosome mass and the cell volume is directly proportional to the chromosome number. On the other hand, Conklin's investigations on annelids, mollusks and ascidians lead him to take a position opposed to that of Boveri. He says:

> The size of the nucleus, centrosomes and chromosomes is dependent upon the volume of the cytoplasm is clearly shown in *Crepidula,* where in large and small blastomeres, these structures are invariably proportional in size to the volume of cytoplasm.

Neither chromosomes nor nucleus control, the size of the cell in annelids, mollusks or ascidians.

Relations between Chromosomes and External Characters

Thus there seems to be no constant relationship even between nuclear or cell size and number of chromosomes, and bonds of union between external taxonomic characters and chromosome number seem to be still more tenuous. It is true that certain giant Primulas and Œnotheras had more chromosomes than were characteristic of the normal forms, but it is just as clear that all giant Primulas (and the same is probably true of Œnotheras, from the work of Heribert-Nilsson and of Geerts) do not have abnormal chromosome numbers.

Results on several species of both animals and plants are interesting in this connection.

The thread worm, *Ascaris megalocephala,* has two varieties, *bivalens* and *univalens,* the former having as a 2N number four chromosomes, the latter two chromosomes. Nothing is known as to the origin of these two forms. They are found parasitic in the same host individual and neither form is rare. According to Herla, they hybridize freely and produce embryos whose cells have three chromosomes, but no mature hybrids have ever been found. Meyer could distinguish no anatomical differences between the two varieties.

Rosenberg investigated the reproductive structures of two species of sundew and found one to have double the chromosome number of the other. A subsequent comparison of anatomical and taxonomic characters failed to show any sharply marked differences between them except in size. The form having the smaller chromosome number was smaller and less robust. They inhabit the same territory and produce natural hybrids which are sterile.

Rosa canina has two varieties which have the same taxonomic characters, but one form has thirty-four while the

other has only sixteen chromosomes. The form with thirty-four chromosomes is apogamous and reproduces without fertilization, but that one must not conclude that apogamy is necessarily associated with a double or an increased chromosome number, is clear from the case of *Rumex*. *Rumex* was investigated by Roth; one species, *R. cordifolius*, having forty chromosomes as its 2N number, required fertilization to produce offspring; another species, with only sixteen chromosomes, was apogamous.

A short list of nearly related species or species with two varieties varying in their chromosome numbers with their character differences, if present, is given below.

Name	Date	N	2N	Characters	Investigator
Alchemilla Eualchemilla	1904	32	64	Apogamous	Strasburger, E.
" *aphanes*	1904	16	32		" "
Ascaris megalocephala	1883	2	4	Alike externally	Van Beneden
" "	1895	2	4		Meyer, O.
" "		1	2		" " and others
Ascaris lumbricoides	1887		24		Boveri, T.
" "	1887		48		" "
Dahlia variabilis	1911	16	32		Ishikawa, M.
" "	1911	32	64		" "
Drosera rotundifolia	1909	10	20		Rosenberg, O.
" *longifolia*	1909	20	40	More robust, etc.	" "
Echinus microtuberculatus	1888	9	18		Boveri, T.
" "	1902	18	36		" "
Helix pomatia	1903	24	48	Alike externally	Ancel, P.
" "	1896	12	24		v. Rath, O.
Nephrodium molle	1908	64	128	None mentioned	Yamanouchi, S.
" "	1908	66	132		" "
Œnothera lamarckiana	1911	7	14		Gates, R. R.
" *gigas* form	1909	14	28	Large and coarser	" " "
Primula sinensis	1909	12	24		Gregory, R. P.
" giant form	1909	12	24	More robust	" " "
" " "	1914	24	48	" "	" " "
Rosa canina	1909		34	Apogamous	Rosenberg, O.
" "	1904	8	16		Strasburger, E.
Thalictrum minus	1909	12	24		Overton, J. B.
" *purpurascens*	1909	24	48	Apogamous	" " "
Zea Mays, "White Flint"	1911	10			Kuwada, Y. "
" " , "Sugar"	1911	12			"

What conclusions can be drawn from these facts? Certain botanists have attempted to connect chromosome doubling with apogamy, as usually the chromosome number in apogamous species is higher than in the normal species of the same genus; but there is no evidence of

apogamy in *Œnothera gigas,* and in *Rumex* the form with the low number of chromosomes is apogamous while the form with the high chromosome number requires fertilization. On account of these exceptions, therefore, it seems probable that the cause of apogamy is deeper than a mere doubling of the chromosomes, even though doubling may usually accompany such a change in reproductive habits.

Variation in chromosome number in the same species has been proposed as a cause of general variation in somatic characters, but the evidence is not clearly in favor of such a theory. In the fern *Nephrodium molle* Yamanouchi found spermatid cells to be of two sorts, those with sixty-six and those with sixty-four chromosomes. This would mean that *Nephrodium* has two gametophyte forms and two sporophyte forms, externally identical, so far as our present knowledge goes, but differing in their chromosome numbers.

Further, sporophytes developing from the prothallia of ferns without the intervention of a sexual process have the N instead of the 2N chromosome number, yet apogamously developed fern sporophytes, except as to chromosome number, are indistinguishable from normal sexually produced individuals of the same species.

Many writers have been tempted to postulate a causal relation between the numerical variation of chromosomes among the species of a genus and the genera of a family and their specific and generic characters. The thirty or more species of Compositæ investigated have shown a remarkable variation in their chromosome numbers, the 2N numbers ranging between six and sixty, and, as is well known, the Compositæ possess an infinite variety of sharply contrasting characters. But the lily family also has an enormous number of characters in its species and genera, and the genus *Lilium,* with its great variety of characters distributed among forty-five species, is typical of the other genera of the family, as far as present investigations go, in having the same chromosome number for

all of its species. Others suggest that the more chromosomes a plant species possesses the greater is its variability. Thus Spillman[3] speaks of the low variability of rye, suggesting its small chromosome number (six or eight) as a possible reason; for maize, having probably from twenty to twenty-four chromosomes, is infinitely more variable than rye. However, Britton's "Manual" selects *Crepis virens* for special mention as an extremely variable species from among the four or five other species listed under that genus, and it is known that *C. virens* has only six chromosomes, while three other species of *Crepis* investigated all have higher numbers. Again, according to Wiegand, the *Canna* has only six chromosomes, yet every gardener is well acquainted with the infinite variety in Cannas.

THE CHROMOSOMES AND VARIABILITY

After a consideration of the above facts, one may well hesitate to state that there is even a high degree of correlation either between variability in chromosome number and general variability, or between high numbers of chromosomes and a high degree of variability in specific characters. On the other hand, it is not certain that the data upon which our discussion is based are relevant to the case in hand. We have discussed a possible relationship between chromosome numbers and species complexity and variability as found in the wild. This is not at all the same thing as discussing the relationship between chromosome number and true variability. It is true that complexity and specialization of plants and animals seem to have no connection with chromosome number, and that within a family a genus or a species profusion of taxonomic characters do not go hand-in-hand with high chromosome numbers. But in these cases our data come from persistent forms. What the actual inherent variability of the protoplasm is in most cases we do not know. *Drosophila ampelophila,* a species with only four chromo-

[3] Six according to Westgate's unpublished data; eight according to Nakao.

some pairs, is considered to be very constant in its characters from the taxonomist's standpoint, yet by careful continued observation Morgan has succeeded in detecting over 130 mutations.

From a strictly mathematical standpoint, it would seem that if other things are equal, variability would take place in proportion to the number of chromosome units. The difficulty is that in no case do we know anything whatever about the relative complexity of any particular chromosome unit. One must infer, however, that the 47–48 chromosomes in man are individually much more complex than the 128–132 chromosomes in the fern *Nephrodium molle*. If this inference be correct there are reasons why alteration in determinants may occur in direct proportion to the number of chromosomes or rather to the mass of chromatin without there being visible somatic variability in the same ratio. Let us construct an imaginary plan for preventing visible variation without preventing change in chromosome determinants. Unquestionably the simplest means is to double the chromosome number. Selecting, for example, a species with four chromosomes, let us suppose that fertilization occurs without a reduction division at some time or other. Then instead of a dual organism with two sets of chromosomes of similar function, one from the male and one from the female parent, there would be a quadruple organism with two sets of similar chromosomes from each parent. Any germinal change which would produce a *new dominant* character would be apparent immediately, but for a recessive change to appear—and these are many times as numerous as the others—it would be necessary to have identical changes occur in two chromosomes. Following out this line of reasoning, it is not hard to see what a great possibility for uniformity there is in further chromosome duplication, provided the actual fact of duplication makes no great change in the organism. That chromosome doubling has no decided visible effect we have seen from the cases already described; and since so many nearly related spe-

cies and varieties have their chromosome numbers in series 1:2:3:4, etc., it seems by no means improbable that what we have imagined above has actually occurred many times. And if one may believe that the event has the result supposed, all the worry about relationships between chromosome number and height of species in the scale of evolution may be eliminated.

NORMAL CHROMOSOME BEHAVIOR AND HEREDITY

The second query, concerning the relation of normal chromosome behavior to the transmission of characters, is much more important than the one just examined, but it can be discussed more briefly. By normal "chromosome behavior" is meant a reduction division where maternal and paternal chromosomes approach each other in definite pairs (if homologous pairs are present), chance only governing the passage of either to a particular daughter cell. This is probably the usual behavior in the higher plants and animals, and upon this behavior depends Mendelian heredity in the narrow sense. The thesis to be submitted and scrutinized is the following: *The maximum possible difficulty in the improvement of animals and plants by hybridization usually depends directly upon the chromosome number.*

When a mutation in a single determinant takes place in the germ cells of a plant, such as may cause the loss of red color in the corolla, crosses between such a form and the normal give a monohybrid Mendelian result. Two mutations in non-homologous chromosomes gives in a similar way a dihybrid result. Such simple conditions, however, are not met with very frequently. For example, White found that a fasciated tobacco when crossed with the type from which it sprang and from which it probably differed only by this single determinant, gave a monohybrid Mendelian ratio in the F_2 generation; but when the fasciated type was crossed with other types the result was a complex F_2 population. This population was susceptible of analysis, nevertheless, and showed that the various

varieties with which the fasciated type was crossed differed from it by several determinants, each of which was transmitted independently *though they every one affected the development of fasciation.* This illustration is not one of a rare phenomenon. It is what geneticists find constantly in their experiments. Presence or absence of a particular character may depend upon the presence or absence of a particular essential determinant, but, given this determinant, sooner or later the investigator finds several other determinants which modify the expression of the character. The existence of these modifiers has been the cause of a great deal of confusion in the analysis of breeding results, but in reality the inheritance is simple. The experience that all investigators who have worked intensively have had with them shows that practically all somatic characters are due to multiple determinants in the germ cells. It merely depends on the relative difference between the germ plasms brought together in crosses, how complex the resulting F_2 populations appear. Since even apparently simple characters are thus due to complex germinal interactions, that results of crosses made for the purpose of improving such intangible things as yield, size, quality, etc., should be complex, is not astonishing. In the comparatively extensive experience that the writer has had in breeding tobacco, maize, peas and beans the wide variability of the F_2 population in crosses between distinct varieties leads him to think that it is extremely common for such varieties to differ qualitatively in *every chromosome.* Furthermore, the relative complexity of the segregating populations is much greater in tobacco than in corn and greater in corn than in peas or beans. What can this mean but that when varieties are found that differ qualitatively in all of their chromosomes, the complexity of the result varies directly with the number of chromosomes present.

In Mendelian inheritance the number of actual types (both homozygous and heterozygous) present in the F_2 population when all are represented is 3^n, and the number

of individuals that must be present to give an equal chance for the presence or absence of an individual of every type is 4^n, where n represents the number of allelomorphic pairs. This being true, if differences in all of the chromosomes are predicated in tobacco and in pea crosses, the maximum number of individuals necessary in the F_2 generation to allow for one reproduction of each of the grandparental forms is 4^{24} in the first case and 4^7 in the second case. It is clear that there is an absolutely overwhelming difference in the difficulty of recovering the grandparental forms in the two examples.

Now this is about what one wishes to do in many plant-breeding problems. It is desired to combine one or two characters from one parent with all of the other qualities of the second parent. And such has been my experience that I believe that this maximum possible difficulty in the operation as predicated by qualitative differences in all of the chromosomes often occurs. There can be no question on these grounds of the importance of determining the number of chromosomes in a species before beginning a complex plant-breeding problem, and thus being able to comprehend the maximum possible difficulties that may be encountered. How greatly these difficulties vary may be seen in the very incomplete list of chromosome counts in common plants that is given below.

Common Name	Scientific Name	N	2N	Date	Investigator
Banana.....	*Musa sapientum*, "dole".	8	"16"	1910	Tischler, G.
"	*Musa sapientum*, "Radjah Siam".....	16	"32"	1910	" "
"	*Musa sapientum*, "Kladi"	24	48	1910	" "
Bean.....	*Phaseolus vulgaris*.....	8	16	1904	Wager, H.
Calla lily....	*Richardia Africana*.....	8	16	1909	Overton, J. B.
Canna.....	*Canna indica*.....	3	6	1900	Wiegand, K. M.
"	" "	8	more than 10	1904	Körnicke, M.
Corn.....	*Zea Mays*, "yellow starchy" "amber rice pop," "black starchy," "golden broach field," "white flint".....	10	"20"	1911	Kuwada, Y.
"	*Zea Mays*, "red starchy," "red sugar".....	9–10	"18–20"	1911	" "
"	*Zea Mays*, early 8-rowed sugar.....	9–12		1911	" "

Common Name	Scientific Name	N	2N	Date	Investigator
"	*Zea Mays*, early sugar...	12		1911	" "
Cotton.....	*Gossypium*," hybrid"...	28	"56"	1903	Cannon, W. A.
"	" , "Egyptian".	20		1910	Balls, W. L.
Currant.....	*Ribes* 2 sp.....	8	"16"	1906	Tischler, G.
Dandelion...	*Taraxacum confertum*...	8	"16"	1909	Rosenberg, O.
" ...	" sp.....	12or13	about 26	1905	Juel, H. O.
Elderberry...	*Sambucus* sp.....	18	38	1909	Lagerberg, T.
Evening primrose ..	*Œnothera grandiflora*....	7	14	1909	Davis, B. M.
Evening primrose...	*O. lamarckiana*.....	"7"	14	1907	Geerts, J. M.
		7		1911	Gates, R. R.
Evening primrose...	*O. gigas*.....	14	28	1909	Gates, R. R.
Fern.....	*Nephrodium molle*.....	64 or 66	128 or 132	1908	Yamanouchi, S.
Flag.....	*Iris squalens*.....	12	24	1900	Strasburger, E.
Hawksbeard.	*Crepis lanceolata* var. *platyphylum*.....	5	10	1911	Tahara, M., and M. Ishikawa.
" .	*Crepis virens*.....	3	6	1909	Rosenberg, O.
" .	*Crepis tectorum*.....	4	8	1905	Juel, H. O.
" .	*Crepis japonica*.....	8	16	1910	Tahara, M.
Lily.....	*Lilium martagon*.....	12	24	1884	Guignard, L.
Lily-of-the-Valley....	*Convallaria majalis* ...	18	"36"	1899	Wiegand, K. M.
Lily-of-the-Valley....	*Convallaria majalis*.....	16	32	1909	Sauer, L. W.
Mulberry....	*Morus alba*, "Shirowase"	17?	40–50	1910	Tahara, M.
"	*Morus indica*.....	14	28	1910	Tahara, M.
Nightshade..	*Solanum nigrum*.....	36	72?	1909	Winkler, Hans
Onion.....	*Allium Cepa*.....	"8"	"16"	1898	Schaffner, J. H.
Peony.....	*Paeonia spectabilis*.....	12	"24"	1893	Overton, E.
Pea.....	*Pisum sativum*.....	7	14	1903	Cannon, W. A.
Persimmon..	*Diospyros virginiana*....		30 or more	1911	Hague, Stella M.
Pine.....	*Pinus laricio*.....	12	24	1899	Chamberlain, C. J.
Rice.....	*Oryza sativa*.....	12	24[4]	1910	Kuwada, Y.
Rose.....	*Rosa* sp.—3 species.....	8	16	1904	Strasburger, E.
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Tomato.....	*Solanum lycopersicum*...	12	24	1909	Winkler, Hans
Tulip.....	*Tulipa Gesneriana*.....	12	24	1901	Ernst, A.
Wake-robin..	*Trillium grandiflorum*...	6	12	1899	Atkinson, G. F.
Wheat.....	*Triticum vulgare*.....	8	16	1896	Körnicke, M.
"	" "	8	"16"	1893	Overton, E.
"	" "	8	"16"	1908	Dudley, A. H.

Among these figures are found four of our most important crops—wheat, tobacco, corn and cotton. They contrast strikingly in their chromosome numbers. Wheat and tobacco, species in which the flowers are naturally self-pollinated, have 8 and 24 chromosomes, respectively,

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of individuals that must be present to give an equal chance for the presence or absence of an individual of every type is 4^n, where n represents the number of allelomorphic pairs. This being true, if differences in all of the chromosomes are predicated in tobacco and in pea crosses, the maximum number of individuals necessary in the F_2 generation to allow for one reproduction of each of the grandparental forms is 4^{24} in the first case and 4^7 in the second case. It is clear that there is an absolutely overwhelming difference in the difficulty of recovering the grandparental forms in the two examples.

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in their gametes. Corn and cotton, species usually cross-pollinated, have 10–12 and 20–28 chromosomes, respectively, in their germ cells. These species all have been under cultivation since before there has been recorded history. Many varieties of each exist. It is not at all improbable that with thousands of years of cultivation and selection under diverse conditions, mutations in most of their chromosomes have persisted. If, then, *improvement* means working on character complexes that involve almost all of the plant functions, it does not seem improbable that the actual difference in the difficulty of improving wheat and tobacco is as $4^8 : 4^{24}$, or about 1 to 4,295,000,000. In like manner corn and cotton compare in the ratio $4^{10} : 4^{28}$, or 1 to 68,720,000,000. And is it not true that modern improvement in most of these crops does involve nearly all the plant functions? Yield in wheat involves number and size of grain, and number of culms, with all that these things include in plant economy; yield of tobacco involves number, size and thickness of the leaves. Quality, a mystical word, is perhaps still more complex. In wheat, it takes in habit of growth of both root and stem and such other characters as go to make up strength and hardiness, thickness of pericarp, size of aleurone cells, and the physical and the chemical character of both endosperm and embryo, as well as their size ratios in regard to each other. In tobacco, it includes thickness and strength of leaf, color, texture and all chemical and physical characters that make for flavor and "burn."

One may say that this is all very well as a theory, but that it is all theory, and ask what support is given to it by practise. I have had personal experience with but two of these four crops. I have worked extensively and intensively with corn and tobacco for some ten years. But I have followed carefully the published experiments in breeding wheat and cotton and have seen several of the more important experiments. *And I may say that it was my observation of the extreme difficulty in the experiments with cotton and tobacco as compared with corn and wheat that led to this theory of the cause.*

In proposing this thesis, the chromosomes have been considered as pairs of freight boats loaded with character determiners, shifted bodily to the daughter cells by internal forces of which we are ignorant. Yet this is not the whole truth. The determiners in particular chromosomes seem to be tied together more or less tightly, but they are not always transferred as one package. They are coupled in their transmission to the next generation, but this coupling is not perfect. Breaks in the coupling occur and there is order and regularity in these breaks. Our knowledge on these matters rests upon the researches of Morgan on *Drosophila,* Bateson on the sweet pea, and Tanaka on the silkworm, so it is not certain whether these are common grounds for this regularity or whether each species has regular laws which control the breaks in correlation. But in either case, these breaks do not interfere with our proposition. They only complicate matters. In most of the cases in *Drosophila,* where they are best known, linkage is comparatively tight, *i. e.,* breaks are somewhat rare; but they may become so frequent as to simulate inheritance from separate chromosomes. In those cases our theory is of no value, but if *Drosophila* is any criterion by which to judge, such conditions are very unusual.

Abnormal Chromosome Behavior and Heredity

The third query concerning the relations of peculiar or unusual chromosome behavior to the transmission of characters may be passed over with a word. In certain insects, for example, bees, wasps, aphids, phylloxerans, etc.. odd sex ratios and attendant complexities have long been known. These have been cleared up more or less completely by cytological studies. They depended upon chromosome behaviors that are not usual in animals or plants. Similar peculiar chromosome mechanisms may be present in many other species. Attention is merely called to the fact that if experiments on any plant species appear to show that its characters do not obey the laws that have been demonstrated for so many types, their

cytological eccentricities should be looked into. In them will probably be found the key to the situation. The Œnotheras may be mentioned as a case in point. Their heredity in many cases is not what would be expected by analogy with other plants. We know that in some ways the behavior of their chromosomes is irregular. Further study will probably show that this is the sole cause of their anomalous heredity.

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REGENERATION POSTERIORLY IN ENCHYTRÆUS ALBIDUS[1]

H. R. HUNT

THE primary object of the following experiments was to determine whether *Enchytræus albidus* can regenerate posteriorly, when cut at regions of the body varying from near the posterior end to near the anterior end. Secondly, an attempt was made to compare the rates of regeneration per day posteriorly at the different levels at which the worms were cut in two.

No experiments have been published in which the capacity of this species to regenerate posteriorly has been tested. Nusbaum ('02; '04) studied the histological processes in the regeneration of the Enchytræidæ anteriorly and posteriorly. He found that regeneration anteriorly does not take place as readily as regeneration posteriorly, and that never more than two or three segments regenerate anteriorly.

The animals used in the present experiments were collected in abundance from the coarse gravel of the tidal zone on the seashore at Cold Spring Harbor, Long Island, New York. Six sets of experiments were conducted. Each of the worms was cut into two pieces, the anterior and the posterior pieces being preserved. The average number of segments in this species is not far from sixty. The regions selected for cutting were such as to give fairly comprehensive data as to the regenerative capacity posteriorly at different levels. In the first set of experiments the worms were so cut as to leave only about eight anterior segments; in the second set about sixteen anterior segments; and in the third set about twenty anterior segments. In the fourth set the cut was made near the middle of the worm; in the fifth about sixteen posterior

[1] Contributions from the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College, No. 260.

segments were removed; and in the sixth eight posterior segments. The worms were anesthetized with chloretone, and the operation was performed under a dissecting microscope. The pieces were placed in small sterilized glass bottles, each containing a strip of filter paper and enough sterilized sea water to keep the animals well moistened. Ten pieces of approximately the same length were kept in a single bottle. Throughout the experiment the bottles, each one stoppered with a cork, were kept in an ice chest to restrict the growth of bacteria. The work was begun early in July, 1913, and was continued until the first of October. At the middle of August it became necessary to carry away from the seashore the material then living. After this, fresh water was used for moistening the worms and cleaning out the bottles. The worms, however, seemed to regenerate as well in the fresh-water as in the salt-water environment. The analysis of the results of the experiments was done in the zoological laboratory of Harvard University.

It was found that the *length* of the regenerated segments, as compared with that of the segments in the adjacent unregenerated part of the worm, was a fairly accurate criterion for determining the number of regenerated segments. To test the accuracy of this criterion, parts of eight worms consisting of the twenty most anterior segments were allowed to regenerate for about eight weeks. Having taken the precaution to determine accurately the number of segments in each of the pieces at the time of the operation, it was easy to determine how many segments had regenerated, for of the total number of segments at the end of the experiment all except the original twenty were, of course, regenerated segments. The result thus obtained was compared in each worm with that obtained by counting in the same worm the number of segments posterior to the point where there was an abrupt change in the length of the segments, that point indicating the region of the cut. Table I gives the data for this comparison. The results show that the method which

was used to determine the number of regenerated segments is accurate to within one or two segments, for it will be noted that the results by the two methods never differ by more than two segments, usually by only one. The worm's body is so short that it was found impracticable to secure exactly eight, sixteen, etc., segments in every piece used in the whole series of experiments.

TABLE I

Number of the Worm	Number of Segments Regenerated	
	Total Number Minus 20	As Determined by Segment Length
1	6	7
2	18	18
3	20	22
4	23	24
5	21	22
6	15	14
7	18	17
8	10	12

The results obtained in each of the six sets of experiments have been condensed, for convenience, and are shown in Table II. In the first vertical column of this table the Roman numerals designate the number of the set of experiments. The horizontal lines corresponding to each of these sets give in succession, (1) the number of segments in the pieces used in the experiments, (2) the number of worms operated on, (3) the number that survived long enough to be observed, (4) the per cent of worms that survived and were observed, (5) the period during which the regeneration took place, (6) the number of segments (0 to 24) regenerated by the surviving worms, (7) the average number of segments regenerated in each set of experiments, and (8) the mean rate of regeneration per day of the worms in each set expressed in segments. This mean rate of regeneration was obtained by first computing the rate of regeneration per day (in segments) for each worm in the set, and then averaging all the results. In some worms the number of segments regenerated was observed twice, several weeks elapsing be-

TABLE II

No. of the Set of Experiments	No. of Segments Removed [1]	No. of Worms Operated	No. of Worms Observed	Percentage Surviving	Period of Observation	Number of Individuals Regenerating from 0 to 24 Segments Each																									Average No. of Segments	Rate per Day in Segments
						0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
I	52	95	6	6	36 days	..	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5.0	.146
					57 "	..	..	..	..	..	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	9.0	
II	44	129	31	24	21–29 "	6	1	2	..	2	..	..	1	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2.9	.207
					34–35 "	..	..	..	..	..	2	..	1	2	3	2	1	1	..	1	1	..	..	..	..	..	..	..	..	..	9.4	
					60–81 "	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	3	..	..	1	..	1	2	..	..	15.8	
III	40	30	10	33	44–59 "	..	..	..	..	..	..	2	1	..	..	..	..	1	..	1	..	..	1	1	..	..	..	2	..	1	14.8	.267
IV	30	110	44	40	33–36 "	1	..	..	..	..	1	4	2	7	5	2	1	1	1	1	1	..	..	..	..	..	..	..	..	..	8.8	.238
					41–49 "	..	..	..	..	..	2	..	..	3	3	1	..	1	1	1	..	..	..	..	..	..	..	..	..	..	9.2	
					72–83 "	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	3	..	2	5	1	1	2	5	..	..	18.7	
V	16	164	51	31	26–31 "	5	3	3	..	6	4	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.2	.129
					40–48 "	..	1	1	..	1	3	2	5	2	1	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	6.4	
					64–77 "	3	..	..	..	..	..	..	..	1	3	1	1	1	..	..	1	1	..	..	1	..	..	..	..	..	11.8	
VI	8	100	18	18	25–31 "	..	4	1	2	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2.8	.084
					62–79 "	..	3	1	..	..	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.7	
Total.....		628	160																													

[1] The average number of segments in the body is about sixty.

tween the two observations, so that the total number of observations recorded in any one set of experiments may be larger than the number of worms observed in the same set.

The results of these experiments are summarized in the graph shown in Fig. 1, where the rates of regeneration per day (expressed in hundredths of the length of one segment) are measured on the axis of ordinates (Y), and the length (in number of segments) of the pieces that produced the regenerated parts, are measured on the axis of abscissæ (X). Since sixty is about the average number of segments in this species, that is the value which has been used in plotting the curve. A mathematical analysis of the rates of regeneration at the different levels shows that the difference in the mean rates of regeneration at any two successive levels is significant. But the temperature of the worms was not carefully controlled, and the periods during which the wounds were healing and the worms preparing to form new segments were included in the computation of the mean rates of regeneration. Therefore, the ratio between the rates of regeneration, as here computed, at any two of the six levels only approximates the ratio which would have been obtained between the rates at these two levels by subjecting all the worms to the same temperature conditions and by using in the computation of the mean rates of regeneration only the periods during which the segments were being formed. The curve *suggests*, however, that the rate of regeneration for the posterior half of the body is proportional, or nearly so, to the number of segments removed. Anterior to the twentieth segment the rate of regeneration decreases. May we not have here a curve depending on two opposing sets of factors; one which tends to increase the rate of regeneration as more segments are removed, the other to decrease the rate? In the latter set of factors the amount of available building material may be the most important element.

The worms seemed to regenerate equally well in a fresh-water or in a salt-water environment. Thirty-one of the one hundred and sixty surviving worms lived for about forty days in a fresh-water environment and regenerated. Twenty-six worms from which the sixteen posterior seg-

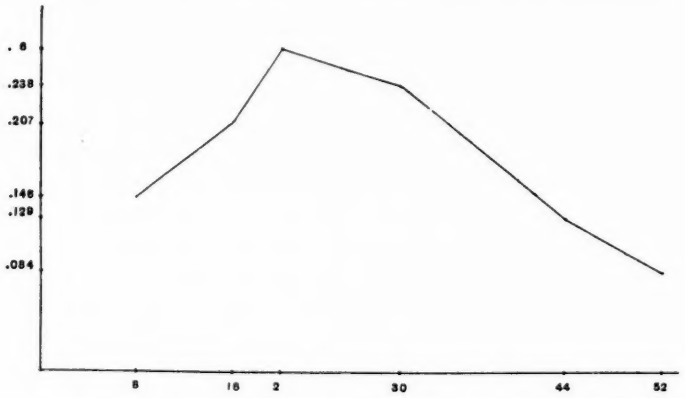

FIG. 1. Curve showing the daily rate of regeneration by pieces of six different lengths. The unit selected to measure the mean rate of regeneration at each of the six levels was 1/100 of a segment, while that used to measure the lengths of the pieces which produced the regenerated segments was *one* segment. In plotting the curve the length selected to represent 1/100 of a regenerated segment (on axis *Y*) was the same as that chosen to represent one segment (axis of *X*) of the pieces producing the regenerated parts.

ments had been removed, and twenty-six others from which the posterior half had been removed, regenerated almost contemporaneously for about thirty days in the same ice chest, and in a salt-water environment. Later in the season in a different ice chest eighteen worms from which the sixteen posterior segments had been removed, and thirteen from which the posterior halves had been removed, regenerated contemporaneously for about forty days in a fresh-water environment. When the sixteen posterior segments were removed the rate of regeneration in the salt-water environment was 0.02 segments per day *less* than in the fresh-water environment, while when the posterior halves were removed the rate of regeneration in the salt-water was 0.07 segments per day *greater* than in

the fresh-water surroundings. These facts show that the worms regenerate in both fresh and salt water. This is not surprising, since individuals of this species are normally found both on the seashore, where they live in a salt-water environment, and also in earth moistened with fresh water. Furthermore, with the exception of the sa-

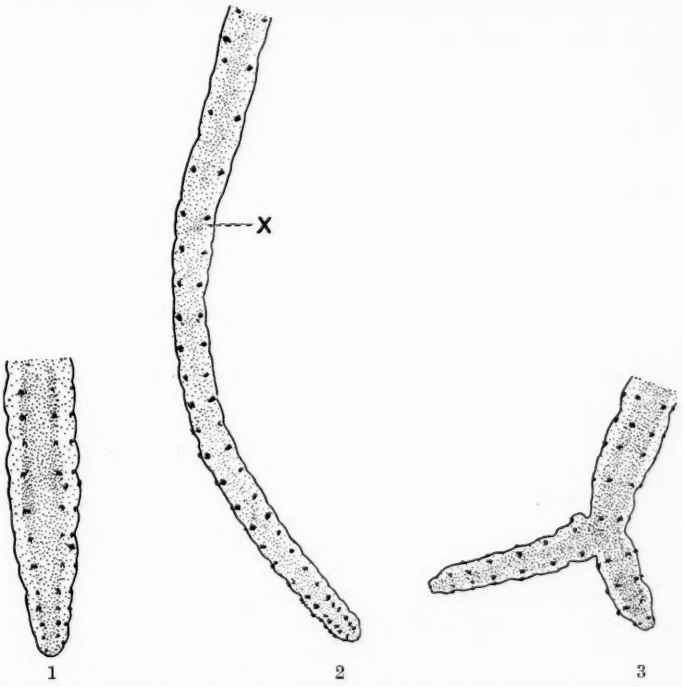

FIG. 2. Camera lucida drawing of the posterior end of a normal worm. Magnified 17 diameters.

FIG. 3. Camera lucida drawing showing the regenerated posterior end of a worm. The region posterior to *X* is regenerated. Magnified 17 diameters.

FIG. 4. Sketch of a regenerated double tail. Magnified about 17 diameters.

linity of the water used to moisten the worms, the worms which regenerated in the fresh-water surroundings were probably subjected to about the same conditions as those which regenerated in the salt-water. Therefore, the salinity of the water in the environment does not seem to affect the rate of regeneration. The data used in plotting

the curve shown in Fig. 1 were secured from worms which regenerated in the fresh-water, as well as from those which regenerated in the salt-water, environment. The above observations make it seem probable, therefore, that the form of the curve does not differ fundamentally from the form which it would have had if all the worms had regenerated in salt-water surroundings.

In. Fig. 2 is shown the normal appearance of the ventral aspect of the posterior end of a worm in which there has been no regeneration. It will be noticed that the length of the segments gradually decreases toward the posterior end; but in Fig. 3, which is a camera lucida drawing of the posterior portion of one of the regenerated worms, the length of the segments decreases abruptly at the point *X*, showing that to be the point at which the tail was removed.

Three worms from which eight posterior segments were removed regenerated double tails. Morgan ('97) and Michel ('98) observed the same phenomena in *Allolobophora fœtida*. One of these worms is shown in Fig. 4.

Some attempts were made to determine the rate of regeneration anteriorly at different levels on the worm's body. At present all that can be said is that regeneration posteriorly takes place much more frequently and rapidly than anteriorly.

The conclusions that follow from these experiments are:

1. *Enchytræus albidus* regenerates posteriorly when cut off at any level between eight segments from the posterior end of the body and eight segments from the anterior end. It will be noticed that although the mortality in pieces containing only the eight most anterior segments was about 94 per cent., yet those that did survive regenerated from three to eleven (on the average seven) segments. In other words, a piece from the extreme anterior end, containing only one eighth the number of segments in the whole worm, can regenerate nearly as many segments, on the average, as it had at the beginning of the experiment. Morgan ('97) found that in *Allolobophora*

fœtida anterior pieces of less than thirteen segments rarely, if ever, regenerate posteriorly. In *Enchytræus* the anterior limit of the capacity to regenerate posteriorly was not found.

2. The rate of regeneration seems to increase from the posterior end of the worm up to its middle almost in direct proportion to the number of segments removed. Anterior to about the twentieth segment the rate decreases.

3. Regeneration can take place either in a fresh-water or in a salt-water environment. Also, the salinity of the water seems to have little or no effect upon the rate of regeneration.

4. Double tails can be regenerated when the eight most posterior segments are removed.

5. Regeneration posteriorly takes place more readily than it does anteriorly.

I am indebted to Professor C. B. Davenport for proposing the problem and for making many helpful suggestions. I also wish to express my gratitude to Professor E. L. Mark and to Professor H. W. Rand for corrections and suggestions in the preparation of the manuscript.

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THE ORIGIN OF BILATERALITY IN VERTEBRATES[1]

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MANY attempts have been made to determine how early in development the vertebrate egg becomes bilaterally symmetrical. The conclusions have been as varied as the attempts.

Before the subject can be discussed it is necessary to consider two fundamental propositions. The first is that there exists an active pole in the egg, and the second is that the anterior end of the embryo develops in this region, or at least in the active hemisphere.

The active pole is indicated at an early period by certain phenomena, such as secretory activity, accelerated yolk metabolism, formation of pigment, position of nucleus, expulsion of polar bodies, etc. Hatschek says that "it is probable that a polar differentiation is present in the unfertilized ova of all the metazoa, through which the most active and least active poles can be determined." Whether or not Hatschek's statement be true, it is certain that if the area in which cleavage grooves first appear be traced backward a differentiation in this area can be found in a very early stage. We are thus enabled to speak of an active pole and an opposite inactive pole. A line passing through the two is designated as the primary ovic axis.

That the active pole or hemisphere gives rise to the embryo was first pointed out by Jan. Swammerdam in his "Bibel der Natur." This view was later supported by Prevost and Dumas, von Baer, Reichert, Cramer, Newport and others. Pflüger, however, believed that the greater portion of the embryo was formed from the inactive hemisphere and his view was supported by Roux, O. Hertwig and others. Most of the later investigators

[1] With observations by C. O. Whitman on Bufo.

including Morgan and Tsuda, Assheton, H. V. Wilson, King, Smith and others have generally agreed that the head end of the embryo forms from the active hemisphere and the caudal portion from the inactive. My own experiments on a considerable number of Amphibia have led to the conclusion that the head of the embryo forms from material which lies at, or near, the active pole of the egg. It thus seems fair to assume that the cephalic portion of the embryo is formed from the active hemisphere.

As stated there have been many attempts to determine how early in development the egg shows bilateral symmetry. Some claim bilateralism for the primitive ovum. Others hold that this condition is not present from the first, but originates at some later period. This period may precede or follow the deposition of the egg. Those who regard the egg as bilaterally symmetrical before deposition claim that this is manifested either through an excentric position of the egg nucleus, or an excentric pigmentation. Those who regard it as fixed after deposition are not in accord. By some the path of the spermatozoon is considered as the determining factor, by others the first or second cleavage groove, and by still others areas of accelerated segmentation.

The assumption that the egg is bilaterally symmetrical from the beginning is based upon nothing more than plausible hypothesis and naturally falls beyond the range of experimental proof.

Some (Schultze) hold that the excentric position of the egg nucleus together with the primary ovic axis determine bilaterality. The work by Roux, Jordan and others, shows that this is highly improbable.

Others (Roux, Morgan and Tsuda) maintain that the excentric arrangement of pigment enables one to determine bilaterality. Professor Whitman's observations which are recorded in a later paragraph, together with his drawings, indicate that the arrangement of the pigment is of significance in *Bufo*. The observations of Moskowski on *Rana*, Morgan's later observations on

Bufo, together with my own on *Amblystoma*, have thrown doubt upon this conclusion.

Still others (Newport, Roux) believe that the path of the entering spermatozoon and the primary ovic axis determine bilaterality. Jordan has shown that this view is untenable for *Diemyctylus*. Professor Whitman's observations, recorded in a later paragraph, show that this is not true in *Bufo*.

Thus each of these assumptions has been met by serious objections.

The idea that the first plane of cleavage determines the axis of the embryo was expressed as early as 1853 by Newport in the following words:

> I have long been aware that the axis of the embryo was in the line of the first cleft of the yolk.

From a series of experiments on the frog's egg Roux came to the conclusion that the first cleavage plane coincides with the median sagittal plane of the embryo. In the same year Pflüger reached the same conclusion. Supported by these eminent investigators the theory was very generally accepted. In working over the same field Rauber found that in the axolotl and frog the median plane of the embryo coincided with the second cleavage groove instead of the first. Shortly after the publication of Rauber's work, O. Hertwig working on the egg of *Triton* confirmed the observations of Rauber. In 1892 Roux modified his earlier view and stated that the second groove as well as the first often coincided with the median plane of the embryo.

In the following April the writer found from a series of puncture experiments on the egg of *Amblystoma* that exovates on opposite sides of the first cleavage groove were later found on one side of the embryo. The conclusion was that in these cases the first cleavage groove did not separate the right and left halves of the embryo.

In 1893 Jordan and the writer reviewed the experiments up to this date. We found that even in the descriptions and figures given by Newport, Roux, Rauber, there was evidence sufficient to show that the median

plane of the embryo often deviated widely from the first or second cleavage planes. We accordingly undertook an extended series of observations on the living segmenting eggs of *Amblystoma, Diemyctylus, Rana* and *Bufo.* Our conclusions were as follows:

> The first and second cleavage planes undergo, even in the earlier stages, extensive torsion. Everything indicates that the extent of this shifting increases greatly in later stages. This led us to conclude that the earlier cleavage planes and the embryonic axes have no vital connection and that the coincidence where it exists is of no fundamental significance.

The later observations by Grönroos, v. Ebner, Morgan and Tsuda, Kopsch and others have likewise emphasized the significance of these variations.

It is scarcely necessary to state that if these cleavage planes mark embryonic areas, the amount of material set apart in different eggs for similar parts of their respective embryos, must be exceedingly variable, and these excesses and deficiencies must be corrected by a corresponding retarded or accelerated growth until the norm is reached, but there is not the slightest evidence that such corrections occur.

These wide variations have been repeatedly observed not only in various amphibia but also in practically all classes of vertebrates: in *Amphioxus* by Wilson; in *Petromyson* by McClure, Kupffer, Eycleshymer; in Dipnoans by Semon; in Ganoids by Salensky, Dean, Whitman and Eycleshymer; in Teleosts by Coste, Hoffmann, His, Agassiz and Whitman, Kingsley and Conn, Clapp, Sobotta and others; in Reptiles by Agassiz and Clark, Oppel, Sarasin; in Aves by Coste, Koelliker, Kionka; in Mammals by Duval, v. Beneden, Assheton, Sobotta and many others.

The inevitable conclusion from such a mass of evidence can not be other than that neither the position or direction of cleavage grooves has the slightest significance as far as the setting apart of definite embryonic areas is concerned.

If then it may be considered an established fact that

neither the position nor the direction of the cleavage grooves enables one to predict the long axis of the embryo, we are naturally led to look for other phenomena which may be of significance. As stated in an earlier paragraph my experiments showed that the head end of the embryo is formed at, or very near, the active pole, and since this area is the one in which cell division is most rapid, it was concluded that the anterior end of the embryo, which is the first to differentiate, was indicated by this increased cellular activity. I accordingly stated that an area of increased cellular activity indicates the position of the head end of the embryo. As is well known, this area can be located with the advent of the first cleavage groove.

While the head end of the embryo may thus be readily located, the median plane of the body may lie in any one of an indefinite number of meridians. The question which now arises is which one of these meridians will represent the median plane of the future embryo.

The writer's studies on *Rana, Bufo, Acris, Amblystoma, Necturus* have shown that in another portion of the egg there is an area of smaller cells, and that this area of smaller cells always marked the region of the forthcoming blastopore. The blastopore in turn definitely fixes the posterior portion of the embryo.

With the recognition of these areas of accelerated cellular activity, the one at the active pole, indicating the position of the future head of the embryo, the other at the side of the egg, indicating the position of the forthcoming blastopore, it necessarily follows that the median plane of the embryo must coincide with a line passing through the centers of the two.

When these observations were first published in 1898, many questioned the existence of such a secondary area of cellular activity. Yet a search through the literature showed that such an area had been observed in many groups of vertebrates. Lwoff found such an area at the posterior end of the embryo of *Amphioxus*. The figures of the segmenting blastodiscs of Elasmobranchs, given

by Balfour, Rückert, Gerbe and Sobotta all show that in these forms such an area is present. In the Reptilia, Vay's studies on *Tropidonotus* show that an area of small cells represents the posterior end of the embryo. v. Koelliker first called attention to such an area in the blastodisc of the chick and suggested that it determines the position of the posterior end of the embryo. The later investigations of Duval and Kionka leave no doubt as to the frequent and probably constant appearance of this area in the locality which later becomes the posterior end of the embryo.

In 1904 the writer made a study of the egg of *Necturus*, which from its size is especially favorable for surface study. This work was undertaken with a view of ascertaining how early this secondary area could be located. It was found that as early as the fourth or fifth cleavage, the cells on one side began to divide more rapidly than any others, excepting those of the primary area. It was possible to predict in this form the median plane of the forthcoming embryo at an extremely early stage of cleavage.

The following year de Bussy from his studies on the Japanese *Cryptobranchus* emphasized the fact that he could find no secondary area of accelerated cell division such as had been described by the present writer. Yet Smith working on the American *Cryptobranchus* says that he finds "an accelerated cell division about a radius of the blastodisc which gives a condition of bilateral symmetry."

The writer felt that it was scarcely necessary to follow the subject further and should not have rehearsed the findings had it not been that certain material came into his hands last year which bears directly upon this subject. This material consists of unpublished descriptions and drawings made by the late Professor C. O. Whitman in June, 1894. These were turned over to me by the department of zoology of the University of Chicago. Professor Whitman's notes run as follows:

Hitherto we have obtained eggs the first week in June. This year we could find none until July 1. We had several night rains, enough to flood the low ground behind Breakwater Hotel. On the evening of June 30, the day after the rain fell copiously, the toads swarmed in this place, and had a carnival of noise; the whole place rang with so many voices as to be almost deafening. On the morning of July 1, we found a great many eggs. The following night the singing followed but much reduced, and only two pairs of toads were captured. The next night the water had gone except in one of the ditches and no toads were to be heard, and of course no eggs. It would seem that rains stimulate them to lay; and the lateness of the season may have been the reason that the egg-laying was confined almost entirely to a single night.

The unfertilized eggs are by some said to be unoriented, that is they are said to be unable to take the normal position assumed by the fertilized egg. The sperm is supposed by some observers to mark the first plane of division and to give the egg the power to right itself. I find that it is not true that the eggs will lie just as they happen to fall, although they do so more nearly before fertilization than after it. If an egg be separated from the rest and turned about for some moments with needles, so as to loosen its adhesion to the membrane, and then rolled to one side so that the equator is vertical, one observes that it slowly turns and in the course of a minute, or sooner, it takes the normal position with the blacker pole uppermost and the whiter showing a little on one side, when viewed from above. This was repeated several times and on several eggs with the same result. The motion is so slow that one does not notice it until after the lapse of some seconds.

I cannot affirm that all unfertilized eggs will right themselves; ordinarily they do not if left to themselves. They assume an irregular wrinkled appearance and have so little power of righting that they stick to the membrane enough to prevent it. When fertilized they contract and round up and get freedom of space to move in. The entrance of the sperm evidently increases the disproportions between the weights of the upper and lower pole. The upper pole becomes lighter and the egg rights itself more readily and quickly. The orientation of the egg is complete before fertilization.

In the eggs which are in the stage of first cleavage there is a small depression which I have found by examination of earlier stages is the "fovea germinativa" of Max Schultze, or the "fosette germinative" of Bambeke. I find further after fertilization, a second point or depression, which probably is the place of penetration of the spermatozoon. The fovea marks the upper pole, but is not placed at the middle of the upper hemisphere; it is excentric.

I followed two eggs which showed both the fovea and the spermatic dent. In neither did the first cleavage plane pass through this dent. In one case it passed far from it while the second cleavage passed near to it. In another case the dent is in the middle of one of the first four

cells, and on the darker side of the upper hemisphere. If this be the sperm track it does not determine the median plane of the embryo.

Cleavage

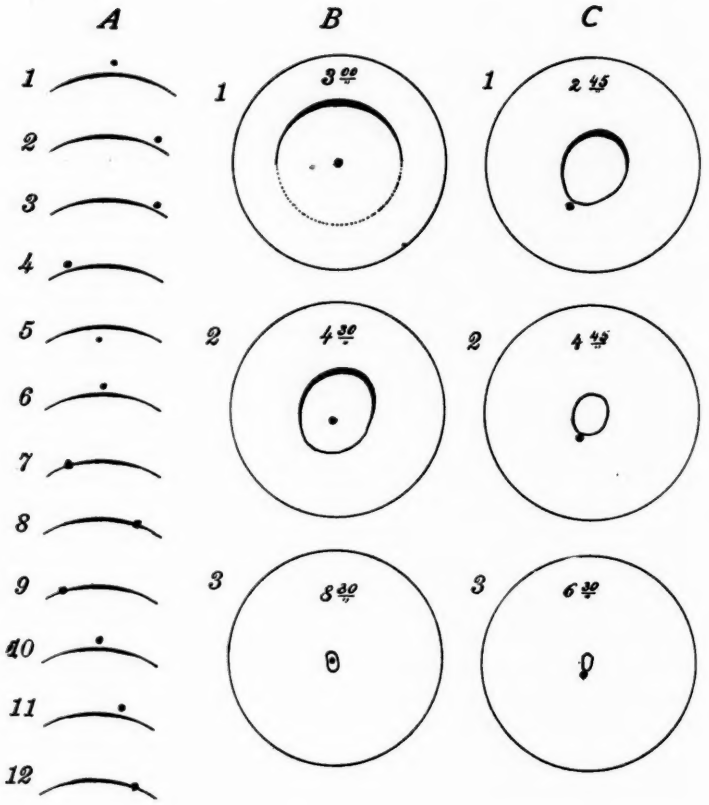
The eggs were obtained in the two-cell and four-cell stages. At this time the pigment is excentric, falling a little short of the equator on the one side and a little beyond it on the opposite. [The notes nowhere state that the antero-posterior direction of the embryo is indicated by the distribution of pigment, yet I think an examination of the figures can not fail to convince all that their interpretation can not be otherwise.—A. C. E.] When the first cleavage groove runs in the plane of symmetry the second cleavage grooves are at right angles and appear at about the same time in both halves as shown in Figs. 3 and 4. When the first cleavage groove is transverse to the plane of symmetry the second cleavage grooves do not appear at the same time, but the one on the lighter side of the upper hemisphere appears first, as shown in Fig. 1. The third cleavage usually cuts off all the pigment into the upper cells on the blastoporic (posterior) side, but leaves considerable below the upper cells on the opposite (anterior) side. The second equatorial usually cuts off all the pigmented cells on the anterior side of the egg and non-pigmented cells on the posterior (blastoporic) side.

The blastomeres on the posterior (blastoporic) side are smaller than on the anterior side, from the very first. It is the blastoporic side that takes the lead in division and the cells are smaller here all the way up to the time when the blastopore appears.

It is thus obvious that the findings by Professor Whitman not only lend confirmation to my observations on bilaterality, but that they in reality anticipate them.

It may be said with added confidence that bilaterality in the vertebrate egg is revealed through the early cleavage grooves. The cephalic portion of the embryo is indicated by the area in which cleavage grooves first appear and in which cellular division is most rapid. The caudal portion is indicated by a secondary area of cellular activity in the blastoporic region. These two areas pass into each other constituting an embryonic tract.

In addition to the above observations, Professor Whitman's manuscript and drawings give the results of a series of puncture experiments in the blastoporic lip. Since these observations have an important bearing on the question of epiboly, emboly and concrescence, they are appended.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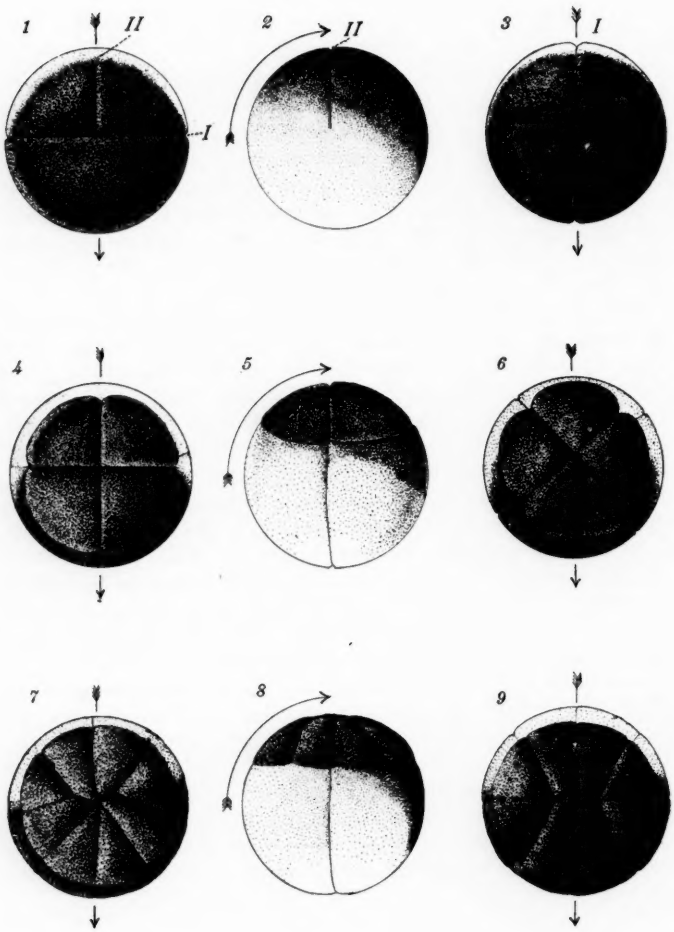
Experiments

On June 5, 1894, sixteen eggs in the thirty-two cell stage were punctured at the equator, in the middle of the white cells, as shown in Fig. 11. In twelve the blastopore appeared near the puncture as shown in the accompanying cut. The extraovates were found in the positions shown in *A*, 1–12, at 10:00 A.M. the next morning. The variations in positions are doubtless due to my punctures falling at different points, sometimes hitting as in Fig. 12, at other times in the very edge of the pigment. In the four remaining eggs two showed no extraovate and two showed no blastopore.

On June 4, 1894, pricked egg *B* at middle of lower pole, soon after the blastopore was sharply marked on the side of the embryo. Ventrally this outline was not clearly marked. At 4:30 this blastopore was outlined all around and nearly circular or about ½ diameter observed at 3:00. At 6:30 the blastopore was far advanced and nearly circular. At 8:30 it was nearly closed. It will be noted that the extraovate remained central throughout.

Another egg *C* was punctured in the ventral edge of the blastoporic rim, and the extraovate was carried along by the closing blastopore. I ought to have made two punctures, one in the middle as well, so that this approach could have been seen. However, my notes show that the blastopore advanced evenly. In this case the extraovate is carried along by this overgrowth, and one might imagine this puncture a fixed point, approached by the blastopore from the opposite side.

June 5. I pricked a number of eggs in the early cleavage stages (8–64 cells) at lower pole. In most of these eggs the extraovates were found after two to three hours to lie at or near the equator of the egg. This was long before the appearance of the blastopore. The extraovate has evidently moved and if one should leave the egg until the blastopore appeared and then look at it, it might be found at the middle of the body; and thus it might appear as if the embryo had lengthened across the lower pole (Roux). Sometimes extraovates have moved and the punctures healed.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Explanation of Plates

Since no explanation of the figures could be found other than those included in the preceding pages, I have endeavored to give an explanation in accord with the text. It should be remembered however that the figures may be open to other interpretations than those presented. The figures show the distribution of pigment and the relation of the embryo and the cleavage planes to the pigment. It will be noted that the eggs when viewed from above show a lighter area or crescent on one side. This excentric position of the pigment is likewise well shown in profile. The arrows in all cases show the direction of the forthcoming embryo.

Fig. 1. Shows the upper hemisphere of an egg in which the embryonic axis is indicated by a line passing through the centers of the light crescent and the more deeply pigmented area. In this case the first cleavage plane (I) passed at right angles to the embryonic axis. It is of interest to note that the second cleavage (II) has appeared in that portion of the egg nearest the grey crescent and further that it coincides with the median plane of the embryo.

Fig. 2. Shows a profile view of an egg in which the first cleavage coincides with the median plane of the embryo while the second is at right angles to the same. I am at a loss to understand the extent of the arrow in this and the succeeding profile views. It may be that Professor Whitman intended thus to indicate the limits of the embryonic anlage.

Fig. 3. Shows the upper hemisphere of an egg in the four cell stage. In this case the median plane of the forthcoming embryo coincides with the first cleavage groove.

Fig. 4. Shows the upper hemisphere of an egg consisting of eight cells. It is to be noted that the formation of the first equatorial sharply separates the lighter and darker portions of the egg on the one side but not on the opposite side. In this case the median plane of the embryo coincides with either the first or second cleavage groove.

Fig. 5. Represents the profile view of either the same egg or another egg in the same stage. In this case the differences in the distribution of the pigment are again shown.

Fig. 6. Shows the upper hemisphere of an egg in which neither the first nor the second cleavage grooves coincide with the median plane of the embryo.

Fig. 7. Shows the upper hemisphere of an egg at a time when the fourth cleavage grooves are present. It is impossible to say whether the median plane of the embryo coincides with either the first or the second cleavages. The appearance of the cleavage grooves leads me to infer that the direction of the arrow is parallel with either the first or the second.

Fig. 8. Represents a profile view of either the same egg or another egg in the same stage.

Fig. 9. Shows the upper hemisphere of another egg in which the fourth cleavage grooves are present. In this egg the median plane of the embryo coincides with the first or second cleavage groove.

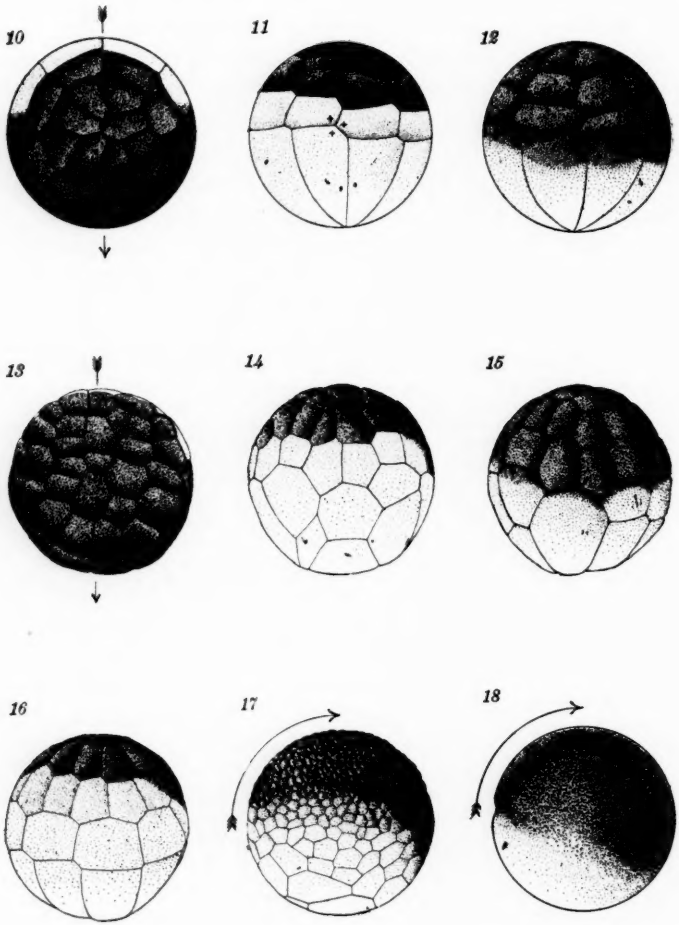
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FIG. 10. Shows the upper hemisphere of an egg in a later stage of cleavage. It should be noted that the lines representing the primary grooves are entirely obscured by a shifting of the blastomeres.

FIG. 11. Represents a profile view of the same (?) egg, viewed from the side on which the blastopore is forthcoming. The small crossed lines represent the localities in which Professor Whitman punctured the eggs of this stage.

FIG. 12. Represents a profile of the opposite side of the same (?) egg.

FIG. 13. Shows the upper hemisphere of an egg in a later stage of cleavage. It should again be emphasized that it would be impossible to trace any one of the primary cleavage grooves. The cells have undergone shiftings to such a degree that if the median plane of the embryo coincided with either of the first two grooves it must be extremely irregular.

FIG. 14. Represents a profile view of the same (?) egg viewed from the side in which the blastopore will later appear. On this side cell division is decidedly in advance of the opposite side.

FIG. 15. Represents a profile view of the opposite side of the same (?) egg.

FIG. 16. ?

FIG. 17. Represents a profile view of an egg in late segmentation. The side in which the blastopore will appear is now indicated not only by the distribution of pigment but also by a decided acceleration in cell division.

FIG. 18. Represents a profile view of an egg at the time when the blastopore appears. The figure shows that it appears on the side of the egg which is least pigmented.

SHORTER ARTICLES AND DISCUSSIONS

THE TORTOISESHELL CAT

IN *The Journal of Genetics* (June, 1913), Doncaster has summarized genetic data dealing with the tortoiseshell cat. The records are collected from fancy breeders and from the work of Dr. C. C. Little.

Aside from certain disputed points the inheritance is in accordance with simple sex-linkage and is analogous to the human defects—color-blindness, night-blindness, nystagmus, and hemophilia, and to the thirty or more sex-linked factors of *Drosophila*.

If the factor for yellow be represented by Y and its allelomorph, the factor for black, by B, the lack of either by b, the sex factor by X, and the allelomorph of X by x, the normal zygotic possibilities are as follows: YX — bx = yellow male. BX — bx = black male. YX — YX = yellow female. BX — BX = black female. YX — BX = tortoiseshell female.

It is obvious then that there can be but two classes of males, while there are three classes of females. Difficulties arise when it is attempted to explain the occurrence of black females produced either by the mating of a black female to a yellow male which should give only tortoiseshell females and black males. or by the mating of a tortoiseshell female to a yellow male, which should give only tortoiseshell and yellow females and black and yellow males. The occurrence of the rare tortoiseshell male is also the cause of considerable difficulty. In one mating out of seventeen of yellow females to yellow males there were produced three tortoiseshell females. There are recorded in addition from the seventeen matings forty yellow females and forty-eight yellow males which are in agreement with expectation.

In order to explain these discrepancies it is suggested that possibly the linkage of Y with X is not absolute. Yellow males may then produce gametes bX and Yx in addition to the normal or more frequent gametes YX and bx. Gamete bX is female determining, while gamete Yx is male determining and yellow bearing. The latter gamete should produce a tortoiseshell male when it meets an egg BX.

On this hypothesis we should expect the tortoiseshell males to be as frequent as the anomalous black females from yellow fathers. From the matings recorded there are eighteen anomalous black females and only three tortoiseshell males, and one of these tortoiseshell males had a black father. There is a fur-

ther objection to this hypothesis inasmuch as it is not explained how gamete bX differs from BX. Doncaster admits these difficulties, stating that further work is necessary before a definite conclusion can be reached.

In a more recent paper[1] Doncaster has suggested non-disjunction of the sex-chromosomes in oogenesis as a possible explanation. This explains the matroclinous black females, but fails to account for the lack of an equal number of patroclinous yellow males. It also fails to account for the tortoiseshell male and the occurrence of tortoiseshell females among the offspring of yellow by yellow.

In a series of experiments begun upon cats at the University of Pennsylvania during the last year, the tortoiseshell problem has been especially investigated. A yellow Persian male was crossed with common cats—black, maltese and tabby. The results, although not at present extensive, are sufficient to explain, at least in part, the anomalies observed, and to suggest a simple explanation for the occurrence of unexpected classes.

When the yellow male was crossed with a maltese female, a maltese male and two blue and cream females were produced. The blue and cream is the maltese or dilute tortoiseshell. When mated to a black female the yellow male produced both dark and dilute kittens. This shows that the black female was heterozygous for dilution. Two of the males were black and two maltese. The two females were dark tortoiseshell. When the yellow male was crossed with a dark tabby, there were produced dark and light tabbies and maltese. Blacks are also to be expected from this mating. The mother is evidently hybrid between tabby and black and between black and maltese. The female offspring showed yellow: the male offspring were without yellow except for tabby striping.

The female offspring obtained from these matings may be arranged in a series, ranging from one that is predominantly yellow to one that is maltese except for a few cream-colored hairs. The maltese with a few cream hairs occurred in the litter of three above mentioned, which included also a maltese male and a maltese female with a small cream patch.

It may be readily understood how a maltese cat with a few cream hairs or its intense form, a black cat with a few yellow hairs, would be recorded as maltese or black, and it is reasonable to suppose that further segregation of distribution factors in the direction of black would have produced a fully black female. This may

[1] *Quarterly Journal of Microscopical Science*, February, 1914.

be compared with conditions in the guinea-pig in which yellow spotting is continuous with total black. The essential differences are that in the cat we have a factor for yellow allelomorphic to a factor for black, that these allelomorphs are sex-linked, and that either alone is sufficient to produce its expected color, but that when one is balanced against the other, as in the tortoiseshell female, other factors governing the relative amounts of the two colors can act and produce continuous variation from yellow to black.

The three tortoiseshell females from the mating of yellow by yellow may be explained by supposing that the mother was gametically a tortoiseshell plus a sum of yellow extension factors and minus a sum of black extension factors.

The occurrence of the tabby factor brings in a restriction of the black pigmentation producing yellow stripes. It is therefore much more difficult to distinguish a tabby from a tabby-tortoiseshell than a black from a tortoiseshell. We have had a few tabby-tortoiseshells that would have been recorded as tabbies if close examination had not been made.

Another source of error in records involving the tortoiseshell pattern may be introduced by the occurrence of white spots. Doncaster makes no mention of these in his paper, so that it is possible that they did not occur in the animals recorded. In what is genetically a tortoiseshell and white cat the incidence of the white spotting may happen to be at just those points which would otherwise be yellow. Thus the occurrence of black and white daughters from yellow males may be explained. It is possible also that the yellow mother of the three tortoiseshell kittens recorded from the mating of yellow by yellow may have been white at points which, if pigmented, would have been black. She would then have been genetically a tortoiseshell and white and some tortoiseshell kittens would have been expected.

I would suggest as a plausible hypothesis that the rare tortoiseshell male is genetically a yellow with an extreme of black extension factors or a black with an extreme of yellow extension factors. This hypothesis is rendered more probable by some slight evidence showing that male tortoiseshells breed like yellows.

There is then no need for assuming in the cat either breaks in sex-linkage or non-disjunction of the sex chromosomes in oogenesis.

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